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A Single Conserved Amino Acid in the Coat Protein Gene of Pea Seed-Borne Mosaic Potyvirus Modulates the Ability of the Virus to Move Systemically in *Chenopodium quinoa*

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Two isolates of pea seed-borne mosaic potyvirus, DPD1 and NY, which both infect pea (*Pisum sativum*) systemically, differ in their ability to move long distance in *Chenopodium quinoa.* DPD1 spreads to uninoculated leaves, whereas NY is restricted to the inoculated leaves. The NY isolate was found to move from cell to cell infecting all parts of the inoculated leaves, including the petiole. The coat protein (CP) coding region was identified as the determinant of long-distance movement. Virus chimeras containing the CP coding sequence of NY were restricted to inoculated leaves, whereas chimeras containing the CP coding sequence of DPD1 infected *C. quinoa* systemically. Mutational analysis of the CP demonstrated that changing the serine at position 47 of the NY CP to proline was sufficient to permit systemic spread of the NY(S47P) mutant. The reverse mutant, DPD1(P47S), in which the proline at position 47 of the CP was changed to serine, was restricted to inoculated leaves. The movement characteristics and CP sequences of 10 additional PSbMV isolates were determined. All isolates caused systemic infection in pea. In *C. quinoa* 6 of the isolates that were restricted to inoculated leaves had a serine at position 47. Two isolates that infected *C. quinoa* systemically had a proline at position 47. Two isolates, S6 and NEP-1, infected *C. quinoa* systemically, but had a serine at position 47 of the CP. This shows that although a proline/serine difference at position 47 of the CP determined systemic spread of the isolates DPD1 and NY, this amino acid alone does not govern the spread of PSbMV in *C. quinoa*.

INTRODUCTION

Systemic infection by vascular movement is conditioned by efficient cell-to-cell movement and the ability of the virus to enter, circulate within, and exit vascular tissues (Seron and Haenni, 1996). Cell-to-cell movement requires one or more virus-encoded proteins such as the 30K MP of tobacco mosaic virus (Deom *et al.,* 1987), the triple gene block proteins (Gilmer *et al.,* 1992; Angell *et al.,* 1996), and the comovirus 58/48K proteins (Wellink and van Kammen, 1989). In addition the coat protein (CP) has been shown to be required for cell-to-cell movement of a number of viruses (Wellink and van Kammen, 1989; Chapman *et al.,* 1992; van der Vossen *et al.,* 1994). For the potyviruses the CP is the only virus-encoded protein that has been shown to be required for cell-to-cell movement (Dolja *et al.,* 1994).

It is generally considered that viruses move long distance as intact virions and the CP is required for vascular movement of many plant viruses (Gilbertson and Lucas, 1996). Only a few viruses, such as barley stripe mosaic virus and tomato bushy stunt tombus virus, produce rapid systemic infection in the absence of the CP (Petty *et al.,* 1990; Scholthof *et al.,* 1993). It has been suggested that virion formation is required for long-distance transport since assembly-deficient CP mutants fail to produce systemic infections (Saito *et al.,* 1990; Heaton *et al.,* 1991; Fuentes and Hamilton, 1993). However, there are examples of assembly-competent CP mutants that fail to spread systemically (Dolja *et al.,* 1994, 1995; Brault *et al.,* 1995), suggesting a specific function of the CP in vascular movement that is distinct from its function in cell-to-cell movement and virion assembly (Dolja *et al.,* 1994). This role of the CP is often host specific, which may reflect a specific interaction between the viral CP and host factor(s) to allow efficient vascular movement (Hilf and Dawson, 1993; Taliansky and Garcia-Arenal, 1995).

In addition to the CP some viruses also encode proteins that provide functions for long distance movement but which are not required for cell-to-cell transport. Examples are the potyvirus HC-Pro (Cronin *et al.,* 1995) and the tombusvirus p19 protein and cucumovirus 2b protein, which promote long-distance movement in a host-specific manner (Scholthof *et al.,* 1993; Ding *et al.,* 1995).

Pea seed-borne mosaic virus is a member of the potyvirus group which belongs to the picornavirus-like supergroup of positive-strand RNA viruses (Koonin and Dolja, 1993). The potyviruses have monopartite genomes encoding a polyprotein that is processed by three virus-

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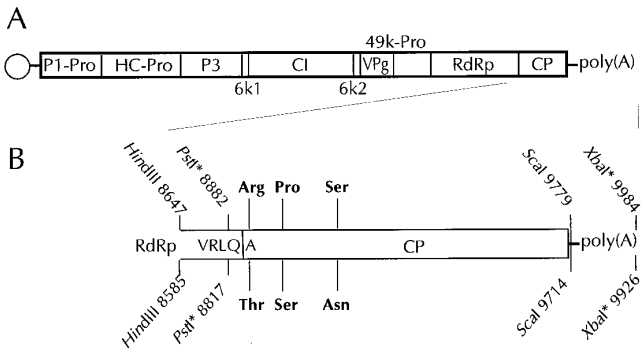

FIG. 1. (A) Cistron map of PSbMV showing the noncoding regions (solid line), the open reading frame encoding the potyvirus polyprotein (open box), and the putative proteolytic cleavage sites (vertical lines). P1-Pro, P1 protease; HC-Pro, helper component protease; P3, P3 protein; 6k1, 6-kDa protein 1; CI, cylindrical inclusion protein; 6k2, 6-kDa protein 2; 49k-Pro, 49-kDa protease; VPg, genome-linked virus protein; RdRp, putative RNA-dependent RNA polymerase; CP, coat protein. (B) C-terminal region of the genome displaying restriction sites used to construct full-length clones. Numbers refer to the first nucleotide of the recognition sequence in the sequence of DPD1 (above the map) and NY (below the map). The *Pst*I* sites were engineered into the sequence at the predicted proteolytic cleavage site, VRLQ ↓ A, between RdRp and CP. To facilitate cloning the *Xba*I* sites were introduced 3′ to the poly(A) tails. The amino acid differences Arg/Thr, Pro/Ser, and Asn/Ser between DPD1 (above) and NY (below) were subjected to mutational analysis.

encoded proteases, the P1 protease, the helper component protease (HC-Pro), and a 49-kDa protease (49k-Pro) (Dougherty and Semler, 1993) (Fig. 1A). Two virus-encoded proteins, the HC-Pro and the CP, have been shown to participate in long-distance movement of tobacco etch virus (TEV), which is one of the best studied members of the potyvirus group (Dolja *et al.,* 1994, 1995; Cronin *et al.,* 1995; Kasschau *et al.,* 1997). Both proteins are multifunctional and in addition to a role in long-distance movement, both are required for aphid transmission. HC-Pro affects virus replication and the central part of CP is required for virion assembly and cell-to-cell movement (Maia *et al.,* 1996; Atreya *et al.,* 1995; Dolja *et al.,* 1994).

Pea seed-borne mosaic virus infection of *Chenopodium quinoa* was originally reported to affect only inoculated leaves while inducing no symptoms on uninoculated leaves (Hampton and Mink, 1975). However, an analysis of two PSbMV isolates, DPD1 and NY, demonstrated that PSbMV isolates differ in their ability to spread to uninoculated leaves of *C. quinoa* (Johansen *et al.,* 1996a). The DPD1 isolate induces chlorotic mottling and distortions on uninoculated leaves and both virus CP and RNA are present in the symptomatic leaves. In contrast no symptoms appear on uninoculated leaves of *C. quinoa* infected with the NY isolate and no virus CP or RNA can be detected outside the inoculated leaves. In this study we have analyzed the spread of the NY isolate and examined the PSbMV genome to identify the coding difference between DPD1 and NY which determines systemic infection on *C. quinoa*.

RESULTS

Cell-to-cell movement of PSbMV NY

PSbMV NY can be detected by enzyme-linked immunosorbent assay (ELISA) in inoculated leaves of *C. quinoa* (Johansen *et al.,* 1996a). To determine if the presence of viral CP resulted from a subliminal infection of primary inoculated cells without further spread or if NY moved cell to cell in the inoculated leaves but was blocked at a later stage in infection, we inoculated NY to one-half of the *C. quinoa* leaves. NY was inoculated to the apical, basal, or lateral half of the leaves. At 1, 2, and 3 weeks postinoculation (wpi) the presence of virus in the inoculated half, the uninoculated half, and the petiole of the leaves was determined by assaying for CP using ELISA and viral RNA using reverse transcription (RT)–PCR employing the NY-specific primers C and D. After 1 week, NY was detected in the inoculated half, but not in the uninoculated half or the petiole. After 2 weeks NY was detected in the uninoculated half of leaves that were inoculated on the basal or apical half. In leaves inoculated on the basal half NY was also detected in the petiole. After 3 weeks, NY was detected in the uninoculated half and the petiole irrespective of which part of the leaf was inoculated (Fig. 2). NY could not be detected at 3–6 wpi in the stem above and below the inoculated leaf, in the stem, petiole, and leaf disc of the sideshoot at the inoculated leaf, in the leaf above the inoculated leaf, or in the young leaves of the plant. *C. quinoa* plants inoculated with DPD1 were systemically infected at 2–3 wpi and displayed extensive chlorotic mottling and distortion of

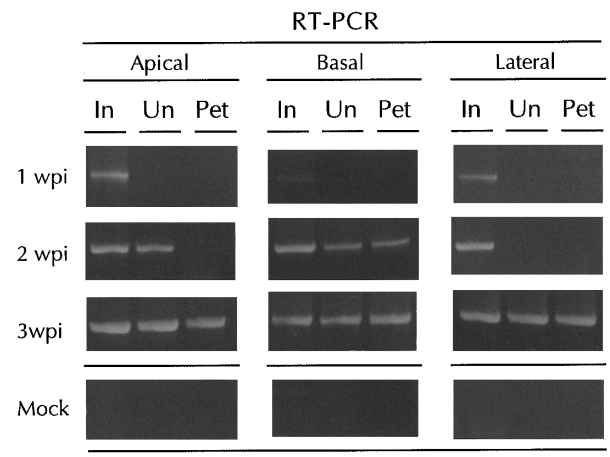

FIG. 2. RT–PCR analysis of local movement of PSbMV NY in *C. quinoa* leaves inoculated on the apical, basal, or lateral half of the leaf disc. The presence of virus was determined by NY-specific RT–PCR in the inoculated half of the leaf (In), the uninoculated half of the leaf (Un), and in the petiole (Pet), 1, 2, and 3 wpi. Mock, mock-inoculated leaf 3 wpi.

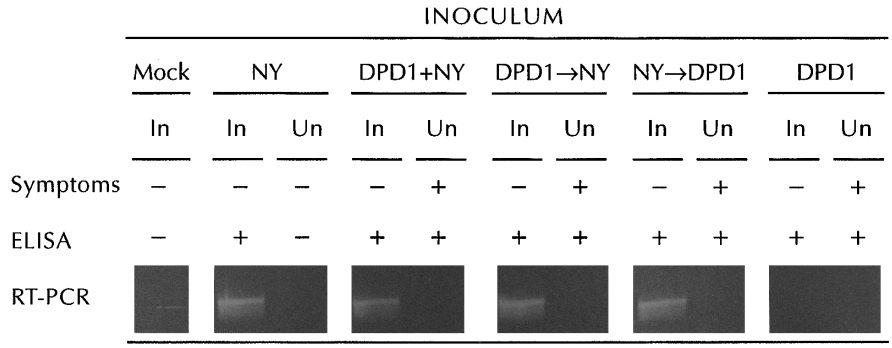

FIG. 3. Coinoculation of *C. quinoa* with DPD1 and NY. Symptoms (+ signifies symptoms typical of DPD1 infection, − no symptoms), the presence of PSbMV CP (+ signifies the presence of DPD1 and/or NY CP measured by ELISA, − no measurable CP), and the presence of NY RNA (NY-specific RT–PCR) were monitored 3 wpi on inoculated leaves (In) and uninoculated leaves (Un) of *C. quinoa.* Plants were inoculated with buffer (mock), NY alone (NY), DPD1 and NY simultaneously (DPD1 + NY), DPD1 first and NY 4 days later (DPD1 → NY), NY first and DPD1 4 days later (NY → DPD1), and DPD1 alone (DPD1).

uninoculated leaves. No symptoms were seen on leaves inoculated with either DPD1 or NY.

Coinfection of *C. quinoa* plants

The restriction of long-distance movement of NY in *C. quinoa* could be due to a defence response elicited upon infection. This putative response would not involve a hypersensitivity as no visible reactions were detected in inoculated leaves. If NY elicited a defence response that limited long-distance movement, this response would probably also restrict DPD1 in coinfection experiments. To test this, *C. quinoa* plants were coinfected with NY and DPD1 or *C. quinoa* plants were first inoculated with NY and 4 days later the NY-infected leaves were inoculated with DPD1 or vice versa. Symptoms typical of a DPD1 infection developed on uninoculated leaves on all plants at 2 to 3 wpi irrespective of the order of DPD1 and NY inoculation. Chlorotic mottling and distortion of the uninoculated leaves appeared simultaneously with the development of symptoms on plants inoculated with DPD1 alone. These data show that coinfection of *C. quinoa* by NY and DPD1 did not result in interference with DPD1 systemic infection and suggest that NY did not elicit a DPD1-limiting defence response.

To test if long-distance movement of NY was complemented by DPD1, the presence of NY in inoculated and uninoculated leaves was assayed using NY-specific RT–PCR with primers C and D (Fig. 3). The NY isolate could be detected only in inoculated leaves but not in uninoculated leaves of the coinfected plants irrespective of the inoculation order. So there was no evidence that DPD1 could complement NY with the long-distance movement factor necessary to spread to uninoculated leaves of *C. quinoa.*

Systemic spread of PSbMV chimeras

Initially four PSbMV chimeras, vP-1114, vP-4111, vP-1144, and vP-4144 (Fig. 4A), were analyzed to determine which part(s) of the PSbMV genome influenced the ability of the virus to infect *C. quinoa* systemically. *C. quinoa* plants were inoculated with sap of pea plants infected with the chimeric viruses. The presence of virus in inoculated and uninoculated leaves was determined by ELISA 3 wpi. PSbMV CP was detected in leaves inoculated with each of the four chimeras vP-1114, vP-4111, vP-1144, and vP-4144, but only in uninoculated leaves of *C. quinoa* inoculated with the vP-4111 chimera. Analysis of the plants as late as 6 wpi gave the same result. The chimeras vP-1114, vP-1144, and vP-4144 were restricted to the inoculated leaves. These chimeras all contain sequences from the 3′ region of NY, which covers the region encoding the C-terminus of RNA-dependent RNA polymerase (RdRp), the CP, and the untranslated 3′-terminus. The chimera vP-1114 contains no other sequences from NY, suggesting that the determinant of long-distance movement of PSbMV in *C. quinoa* is located in this part of the PSbMV genome (Fig. 4A).

Systemic spread of PSbMV CP chimeras

To investigate the 3′ region of the genome further, full-length clones were constructed in which the CP coding region was exchanged. The resulting full-length clones p35S-DPD1(CP-NY) and p35S-NY(CP-DPD1) were infectious on pea, and both viruses produced systemic infection in pea. The viruses DPD1(CP-NY) and NY(CP-DPD1) were propagated in pea and inoculated to *C. quinoa*. At 3 wpi the spread of virus infection was determined by testing inoculated and uninoculated leaves for the presence of PSbMV CP and viral RNA by ELISA and RT–PCR, respectively. The chimeras DPD1(CP-NY) and NY(CP-DPD1) both infected the inoculated leaves of *C. quinoa,* and NY(CP-DPD1) was also present in uninoculated leaves. DPD1(CP-NY) could not be detected in uninoculated leaves by ELISA or RT–PCR (Fig. 4B). Like the parental viruses, the chimeras DPD1(CP-NY) and NY(CP-DPD1) did not induce any visible symptoms on

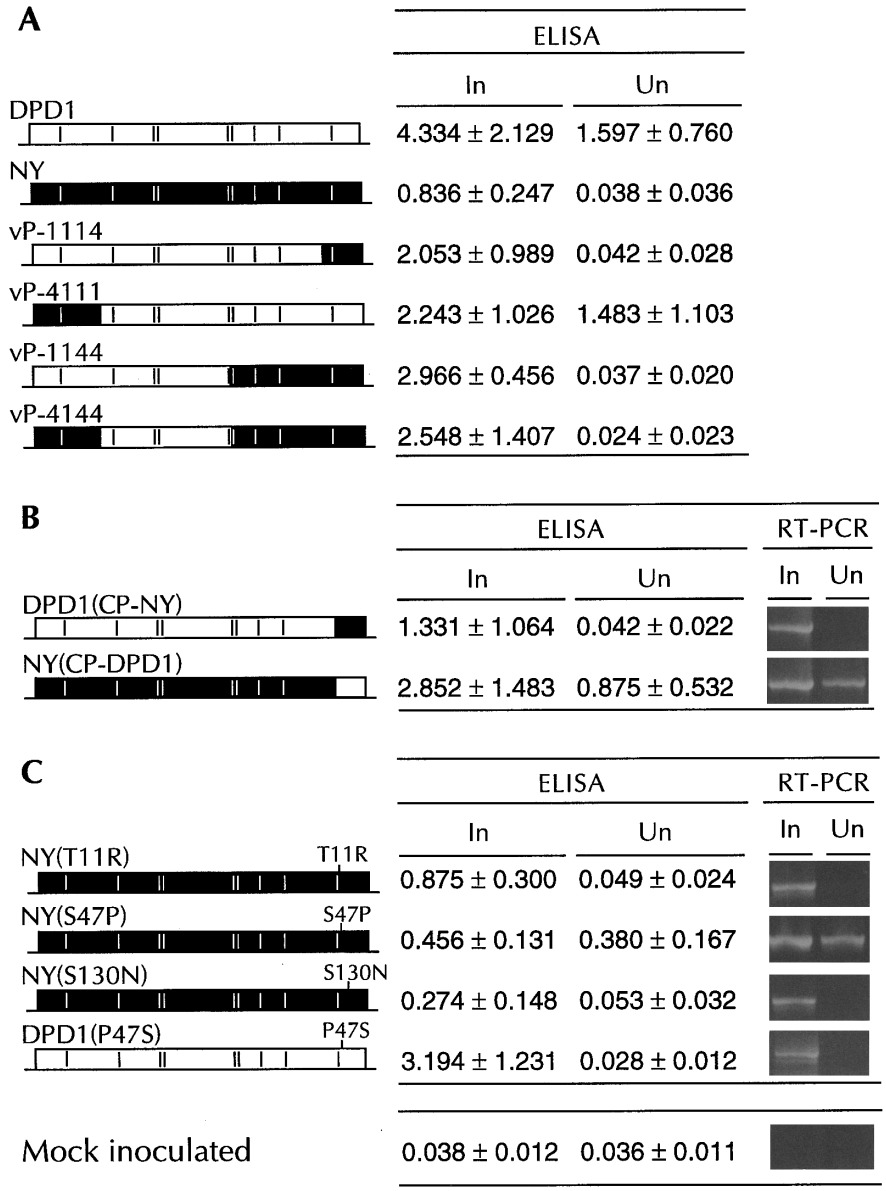

FIG. 4. Infectivity and systemic spread of PSbMV isolates DPD1 and NY and PSbMV chimeras and mutants on *C. quinoa.* Cistron maps of the viruses display DPD1-derived sequences in white and NY-derived sequences in black. Infectivity was measured by ELISA (A_{405} values, mean of six plants ± $LSD_{.95}$) and RT–PCR on inoculated (In) and uninoculated (Un) leaves. Primers A and B were used for PCR of DPD1(CP-NY) and DPD1(P47S). Primers C and D were used for PCR of NY(CP-DPD1), NY(T11R), NY(S47P), NY(S130N), and mock-inoculated plants. (A) Infectivity and systemic spread of PSbMV isolates DPD1 and NY and PSbMV chimeras vP-1114, vP-4111, vP-1144, and vP-4144. (B) Infectivity and systemic spread of PSbMV CP chimeras DPD1(CP-NY) and NY(CP-DPD1). (C) Infectivity and systemic spread of PSbMV mutants NY(T11R), NY(S47P), NY(S130N), and DPD1(P47S).

inoculated leaves. NY(CP-DPD1) gave rise to chlorotic mottling on the uninoculated leaves but without the leaf distortion which was observed on systemically DPD1-infected leaves.

Systemic spread of PSbMV CP mutants

The predicted CP coding region of PSbMV encodes a protein of 301 amino acids. There are 12 amino acid differences between DPD1 and NY within the N-terminal 130 amino acids (Fig. 5). Three of these differences, at positions 11, 47, and 130, which were likely to be of structural or functional significance, were chosen for further analysis (Bordo and Argos, 1991). Three NY mutant full-length clones were constructed, p35S-NY(T11R), in which the threonine codon at position 11 was changed to an arginine codon, p35S-NY(S47P), in which the serine codon at position 47 was changed to a proline codon, and p35S-NY(S130N), in which the serine codon at position 130 was changed to an asparagine codon. Virus derived from the three mutant full-length clones produced systemic infection on pea. Infected pea sap was used to infect *C. quinoa* with the mutant viruses

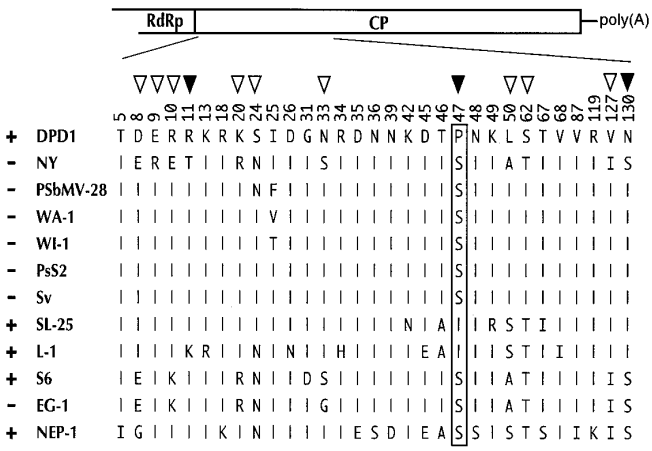

FIG. 5. Variable amino acids in the N-terminus of the PSbMV CPs. Numbers signify the position of the amino acids in the CP of PSbMV. Amino acids not shown are identical in all isolates. Amino acids identical to DPD1 are marked with a vertical line. The positions of amino acid differences in the CP of DPD1 and NY are marked with arrowheads. The black arrowheads mark amino acid differences between DPD1 and NY at positions 11, 47, and 130 which were subject to mutational analysis. Amino acid 47 is boxed. PSbMV isolates which infected *C. quinoa* systemically are marked with +; isolates which were restricted to inoculated leaves are marked −.

NY(T11R), NY(S47P), and NY(S130N). At 3 wpi all three mutant viruses were detected in inoculated leaves of *C. quinoa* by both ELISA and RT–PCR, but only NY(S47P) was also detected in uninoculated leaves. NY(S47P) induced chlorotic mottling of the uninoculated leaves identical to the symptoms induced by NY(CP-DPD1). None of the mutants induced visible symptoms on inoculated leaves.

To test if the proline at position 47 of the DPD1 CP was absolutely required for systemic spread of DPD1 in *C. quinoa*, a DPD1 mutant full-length clone was constructed in which the proline at position 47 was changed to serine. Virus derived from the full-length clone p35S-DPD1(P47S) produced systemic infection on pea. On *C. quinoa* this mutant was detected in inoculated leaves by both ELISA and RT–PCR but could not be detected in uninoculated leaves. The results are summarized in Fig. 4C.

Systemic spread and CP sequence of PSbMV isolates

To determine if there was a correlation between the amino acid sequence of the CP and the ability of PSbMV to move long distance and infect *C. quinoa* systemically, 10 additional PSbMV isolates of different geographic origins were tested. Each isolate was multiplied in pea and inoculated to *C. quinoa*. At 3 wpi inoculated and uninoculated leaves were tested for the presence of virus by ELISA and RT–PCR. All isolates were detected in inoculated leaves and four isolates, SL-25, L-1, S6, and NEP-1, could also be detected in uninoculated leaves. Of these SL-25 and NEP-1 induced symptoms similar to those of DPD1, L-1 gave rise to systemic necrotic lesions, whereas S6 induced few scattered small chlorotic lesions. The S6 isolate was not found in uninoculated leaves of all the *C. quinoa* plants inoculated with this virus. Furthermore, in plants in which the S6 isolate was found in uninoculated leaves, not all samples were positive, suggesting that the S6 isolate did not spread as efficiently as DPD1, SL-25, L-1, and NEP-1 in *C. quinoa.*

To determine the sequence of the variable N-terminus of the CP of these PSbMV isolates, RNA of SL-25, L-1, S6, and NEP-1 was extracted from systemically infected *C. quinoa* leaves. RNA of the rest of the isolates was extracted from the inoculated leaves. The cDNA sequence analysis (Fig. 5) showed that the six isolates which were restricted to inoculated leaves had Ser at position 47 like NY. Of the isolates which infected *C. quinoa* systemically, SL-25 and L-1 had Pro at position 47, as did DPD1, whereas the two isolates S6 and NEP-1 had Ser at position 47.

Virus particles in *C. quinoa*

Long-distance movement of plant viruses has been found to correlate with the ability of the virus to form particles (Fuentes and Hamilton, 1993; Saito *et al.,* 1990; Vaewhongs and Lommel, 1995). It was therefore possible that the failure of some of the PSbMV isolates, chimeras, and mutants to spread from inoculated leaves to uninoculated leaves was due to an inability of these viruses to form particles in this host. To test this, extracts of inoculated leaves of *C. quinoa* were examined by serologically specific electron microscopy (SSEM). All PSbMV isolates, chimeras, and mutants formed typical rod-shaped flexuous particles in *C. quinoa* (data not shown). There is therefore no evidence that failure to form particles in *C. quinoa* was the cause of the restricted movement of some of the PSbMV variants.

A comparison of the ELISA values of the PSbMV isolates and mutants showed that the viruses accumulated to different levels in the inoculated leaves of *C. quinoa,* with DPD1 and NY(S130N) representing the extremes. However, there was no correlation of virus accumulation and systemic spread because DPD1(P47S), which was restricted to the inoculated leaves, gave rise to ELISA readings that were not significantly lower than those of DPD1, whereas NY(S47P), which moved to the uninoculated leaves, gave readings similar to those of NY (Fig. 4).

DISCUSSION

Two potyvirus proteins have been shown to be required for long-distance movement of TEV in susceptible hosts, the HC-Pro and CP (Cronin *et al.,* 1995; Dolja *et al.,* 1994, 1995; Kasshau *et al.,* 1997). Here we have studied the long-distance movement of another potyvirus, PSbMV, in *C. quinoa*. PSbMV was originally reported to infect only inoculated leaves of *C. quinoa* (Hampton and

Mink, 1975). However, of the 12 PSbMV isolates we have analyzed, 5 produced systemic infection, while 7 could not be detected outside the inoculated leaves. Inspection of the ELISA values showed that the isolates accumulated to different levels in the inoculated leaves, but virus accumulation was not correlated with the ability of the viruses to systemically infect *C. quinoa* (Fig. 4). Analysis of the NY isolate, which was restricted to the inoculated leaves, showed that this isolate spread throughout the inoculated leaves and even into the petiole (Fig. 2). There was therefore no evidence that impaired virus accumulation or cell-to-cell movement caused the restriction of some PSbMV isolates to the inoculated leaves of *C. quinoa*.

Molecular analysis of the PSbMV isolates DPD1, which infected *C. quinoa* systemically, and NY, which was restricted to inoculated leaves, showed that the genetic determinant controlling long-distance movement in *C. quinoa* was a single Pro-Ser difference in the CP. In pea, long-distance movement was not affected by this amino acid difference. The amino acid difference was located at position 47 in the variable N-terminus of the CP. Dolja *et al.* (1994) found that the TEV ΔN variant, in which 27 amino acids were deleted from the N-terminus of the CP, was unable to move systemically in tobacco. A comparison of the CP amino acid sequences of TEV and PSbMV revealed that amino acid 47 of PSbMV lies within a region of the CP equivalent to the 27 amino acids deleted from the ΔN variant of TEV.

The surface-exposed N-terminal of the CP could serve as an elicitor of a defence response preventing systemic infection, as seen with the 3a protein of brome mosaic virus in cowpea (Mise and Ahlquist, 1995). This, however, seemed unlikely because no reaction was visible on the NY-inoculated leaves of *C. quinoa*. In addition coinfection experiments showed that the NY isolate did not prevent the systemic spread of the DPD1 isolate, as would have been expected if NY induced a general defence response. It is therefore possible that the potyvirus CP is required to interact with host components in a specific manner to allow efficient long-distance movement and that the CP of the NY does not function in this manner in *C. quinoa*. In coinfected plants the DPD1 CP did not complement NY with the long-distance movement function necessary to infect *C. quinoa* systemically. This could mean that the long-distance movement function of CP must be provided in *cis* or possibly that no cells of the coinfected leaves became infected with both viruses. The mechanical inoculation procedure would not assure coinfection at the time of inoculation but since both viruses move from cell to cell in the infected leaf it would be expected that they would reach the same cells of the coinfected leaves. However, a cross-protection mechanism may prevent the two related viruses from entering or replicating in the same cell.

Sequence analysis of 10 more PSbMV isolates, of which 4 infected *C. quinoa* systemically and 6 were restricted to the inoculated leaves showed that a proline at position 47 of the CP is not absolutely required for PSbMV to move long distance in this host. Both the S6 and the NEP-1 isolates, which were able to move a long distance in *C. quinoa*, had a serine at position 47, as did the NY isolate and all the other 6 isolates that were restricted to the inoculated leaves. The presence of a serine at position 47 of 2 isolates that infect *C. quinoa* systemically suggest that the serine/proline at position 47 may not itself interact with a host factor but could affect long-distance movement through a change in secondary structure of the CP. A closer inspection of both the primary and the predicted secondary structures of the variable N-terminus of the CP did not offer an obvious explanation of the unexpected behavior of these 2 isolates (data not shown). In addition to the N-terminus of the CP, it has been shown that the C-terminus of the CP (Dolja *et al.,* 1994) and the HC-Pro (Cronin *et al.,* 1995) also have functions in long-distance movement of the potyvirus TEV. It is therefore possible that the viral factor(s) in the isolates S6 and NEP-1, which compensated for the proline at position 47 of the CP, could be located in other parts of the potyvirus genome.

MATERIALS AND METHODS

Viruses and plants

The PSbMV isolates used in this study were DPD1 and NY (Johansen *et al.,* 1996a), PSbMV-28 (Wang *et al.,* 1993), WA-1, WI-1, PsS2 (Hampton *et al.,* 1981), Sv (Khetarpal *et al.,* 1990), L-1 (Alconero *et al.,* 1986), S6 (Ligat and Randles, 1993), SL-25, EG-1, and NEP-1. The isolates SL-25, EG-1, and NEP-1 have not been described in the literature before and were kindly provided by R. O. Hampton and S. E. Albrechtsen.

The isolates DPD1 and NY (Johansen *et al.,* 1996a) were used to generate all virus chimeras and mutants. The assembly of infectious full-length clones of DPD1 and NY and construction of the four chimeras vP-1114, vP-4111, vP-1144, and vP-4144 have been described previously (Johansen, 1996; Johansen *et al.,* 1996b). *Pisum sativum* L. cv. Fjord was used to maintain and amplify all PSbMV isolates. All experiments involving *C. quinoa* Willd. employed the genotype designated GCRS. Inoculation of pea with full-length infectious clones or *in vitro* synthesized transcripts was done as described previously (Johansen, 1996). *C. quinoa* plants were inoculated with sap from infected pea plants because the infectivity of transcripts and chimeric full-length plasmids was not always 100%.

Virus infection was routinely determined 2–3 wpi by ELISA (Johansen *et al.,* 1996a). The antiserum, which was raised against CP of the NY isolate, reacts with the CP of all PSbMV isolates used in this study (data not shown). DPD1 and NY isolate-specific RT–PCR was used as

described by Kohnen *et al.* (1992, 1995). An oligo(dT) primer was used for all RT reactions. The primers A (5′-TGTGTATGGTTTTGATCCATGTGAAT-3′), identical to nucleotide (nt) 6179–6204 of DPD1, B (5′-TGTCCTGGGCAGCGGGCAACCACT-3′), complementary to nt 7370–7393 of DPD1, C (5′-TGCGTGAAGGTTAAACAAAACAGTAG-3′), identical to nt 703–728 of NY, and D (5′-ATTCAAGTCCTGTATTGCCAGATGT-3′), complementary to nt 1530–1554 of NY, were used for PCR. Primers A and B are DPD1 specific and give rise to a 1214-basepair (bp) PCR product (Kohnen *et al.,* 1992). Primers C and D are NY specific and give rise to a 851-bp PCR product (Kohnen *et al.,* 1995). SSEM (Derrick, 1973) was used to test the ability of native PSbMV isolates, chimeras, and mutants to assemble into virus particles in inoculated leaves of *C. quinoa*. Semipurified virus particles were prepared according to the protocol of Alconero *et al.* (1986) omitting the final CsCl gradient centrifugation.

Construction of PSbMV chimeras and mutants

All chimeric and mutant viruses were assembled using the infectious full-length clones p35S-P1-IB/IIC/VIA of DPD1 and p35S-P4-VA of NY as base vectors (Johansen, 1996). The CP coding regions of DPD1 and NY were modified on cDNA cloned in pUC118 extending from a common *Hin*dIII site (at nt 8647 of DPD1, nt 8585 of NY) to an *Xba*I site 3′ to the poly(A) tail (Fig. 1B). After introducing the desired modifications into the subclones, the *Hin*dIII/*Xba*I fragments were reinserted into p35S-P1-IB/IIC/VIA or p35S-P4-VA. All mutations were generated *in vitro* by oligonucleotide-directed mutagenesis (Sculptor IVM, Amersham) on single-stranded DNA templates and confirmed by sequencing using Sequenase 2.1 (United States Biochemical).

To construct CP-chimeric full-length clones p35S-DPD1(CP-NY) and p35S-NY(CP-DPD1), a *Pst*I site was introduced in the sequence encoding the LQ dipeptide of the predicted proteolytic cleavage site VRLQ↓A between the putative RdRp and the CP using the oligonucleotides 5′-CAATCAAGGTTCGACTGCAGGCTGGTGACGAA-3′ for DPD1 and 5′-CGATTAAAGTGAGGCTGCAGGCTGGTGATGAG-3′ for NY (the changed nt′s are shown in bold). The CP coding regions were exchanged using the introduced *Pst*I sites and a common *Sca*I site located in the 3′ untranslated region 15 nt's downstream the TAA stop codon of the predicted polyprotein coding region (Fig. 1B).

Three mutants of PSbMV NY with single amino acid changes in the N-terminus of the CP were constructed. The Thr codon at position 11 of the CP was changed to an Arg codon using the oligonucleotide 5′-GAAAGAGAAAGGAGAAAAGAAG-3′; the Ser codon at position 47 was changed to a Pro codon using the oligonucleotide 5′-GAGTGATACACCGAACAAAGCC-3′; and the Ser codon at position 130 was changed to an Asn codon using the oligonucleotide 5′-GATTGGTGACAACGAGATGCAAG-3′. The resulting full-length clones were designated p35S-NY(T11R), p35S-NY(S47P), and p35S-NY(S130N). One mutant of PSbMV DPD1, in which the Pro codon at position 47 of the CP was changed to a Ser codon, was generated using the oligonucleotide 5′-GAGTGACACATCAAACAAATTAAT-3′. The resulting full-length clone was designated 35S-DPD1(P47S). The amino acids affected by mutation are shown in Fig. 1B.

Sequence determination and analysis

cDNA of the PSbMV genome encoding the variable N-terminus of the CP was synthesized by RT followed by PCR using MuLV reverse transcriptase and *Taq* polymerase according to the manufacturer's recommendations (GIBCO BRL). PSbMV RNA extracted from semipurified virus particle preparations was used as template for oligo(dT)-primed RT reactions. Two degenerate oligonucleotide primers were used for the PCR reactions. The 5′ primer, 5′-GC(GATC)ATGAT(ATC)GA(GA)GC(GATC)TGGGG-3′ was identical to a conserved region in the RdRp coding region corresponding to nt 8637–56 of DPD1 and the 3′ primer, 5′-CCG(GA)TT(TC)TC(GAT)AT(GA)CACCA(GATC)ACCAT-3′, complementary to a conserved region in the central part of the CP coding region corresponding to nt 9306–28 of DPD1. The PCR fragments were cloned in pGEMT (Promega) prior to sequence determination. The sequences were analyzed using the Lasergene program (DNASTAR, Inc.) and PepPlot (Genetics Computer Group, Inc.)

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